THEORETICAL STUDIES ON LIVING SYSTEMS IN THE ABSENCE OF MECHANICAL STRESS +

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The law of universal gravitation states that every mass attracts every other mass. In the presence of the earth's gravitational field, therefore, all elements of a living system are attracted to the earth. If, in fact, they do not fall, it is because they are supported in some way. This support is in the form of a mechanical stress, set up by the intermolecular forces in response to the distortion produced by gravity. In conditions of free fall, such as are found in an orbiting vehicle, all the elements in a cell are falling in exact response to gravity. Thus there is no distortion produced, and so there is no mechanical stress. The question arises as to whether the fact that the cell has evolved in the presence of such a stress due to gravity will also mean that in the absence of gravity there will be differences in the cell which affect its living behavior. It is our purpose to see whether there are any reasonable theoretical predictions which suggest an answer to this question.

The condition of free fall is often referred to as

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weightlessness, which is probably a suitable term. It is objected to by some on the grounds that the earth's attraction is still present. By using "absence of mechanical stress" in the title, some may be made less critical. It should be clear, in any event, that the condition we are examining is that of free fall.

The advent of space travel has sharpened the interest in a study of living systems in the "weightless" condition. Such studies are very hard to conduct, except in the practical circumstances of an orbiting satellite. Nevertheless, some studies have been made in which the reduction of a gravity effect has been achieved by systematic rotation of the object, or by use of flotation. Neither of these actually remove mechanical stress, but both may be of great significance in considering the reaction of a developed system to a directional force. A very useful review of this topic has been given by Gerathewohl (1).

It is the intent of this paper to provide background to make it possible to examine the wisdom of sending up relatively small systems in satellites to study the effect of weightlessness. Even though small systems occupy very little space and are not an appreciable contribution to the payload, they may still prove to be abnormally expensive experiments. This is true particularly since the environment in a spacecraft is often not as easily controlled as in the laboratory system. As an example we may quote temperature and radiation uncertainties. In view of this, and in view of the fact that any one culture in the laboratory can exhibit abnormalities, it is questionable whether experiments which seem to be very unlikely

of success should even be attempted. The minimum cost of any experiment in a spacecraft is certainly in the $100,000 range, and this means an experiment should be considered with great care. Because of this fact, an analysis has been made of the reasonable effects to be observed when we consider the physical factors which operate in cells, and particularly those which will operate when there is less than 1 g.

The conclusions reached are that the combination of Brownian movement and the effect of gravity is such as to cause a uniform distribution of almost all components in very small cells. Thus it would not be wise to conduct experiments with bacteria. On the other hand as soon as the dimensions of the cell reaches 10 microns, such categorical statements cannot be made. It looks unlikely that the effects will be observed, but it does not look as though they would be impossible to detect. Finally, studies on developing systems which have dimensions greater than one millimeter seem to be very promising. It would be confidently felt that quite interesting results could be obtained on developing plants or on embryologically developing systems.

Operation of the cell in relation to gravity

In the latter part of the twentieth century it is natural for us to consider single cells as the starting point for thinking about living things. A great deal of insight into cells has recently been gained from an intensive study of the bacterial cell, and for the beginning we will consider the working of such a relatively simple living system.

From the point of view of permanence, the most important

unit in the bacterial cell is its DNA. In a representative cell the mass of DNA, probably organized as two units, is 7.5×10^{-15} gram--each unit being 1300 microns long. This DNA has a two fold action. It makes more of itself and it makes "messenger" RNA. The word "make" refers to the process of fastening small units (nucleoside triphosphates) into place at a location determined by a primer DNA and by an enzyme--the polymerase. The messenger RNA, in turn, is attached to a set of ribosomes and there functions to make protein--again by fastening into place amino acids which are held on to specific "soluble" RNA units. Once the protein is made, a part of it, the enzymic protein, acts as catalyst for a large number of cellular reactions.

A part of the enzymic process is the formation of cell membrane, which is a bimolecular array of lipoprotein.

Distribution under gravity

In the presence of a force field there is a constant conflict between random molecular motion, or Brownian movement, and the ordered distribution of particles under the one directional action of gravity. The distribution which results is familiar in, for example, an atmosphere, and was carefully studied by Perrin in the early twentieth century. If the average density in the cell is taken to be unity (1 gram per cc) and the density of a particular unit is ρ, then for a unit of volume v the difference between the downward pull of gravity and the buoyant upthrust (due to mechanical stress) is $v(\rho - 1)g$, where g is the acceleration of gravity. The average energy of thermal agitation is kT

where k is Boltzmann's constant and T is the absolute temperature. The resulting statistical distribution is

$$\frac{n}{n_o} = e^{-v(\rho-1)gh/kT} \qquad (1)$$

where n_o is the number per unit volume at a certain level and n is the number per unit volume at a height above that level.

In a bacterial cell, h cannot really exceed 1 micron or 10^{-4}cm; kT, for 27°C or 300°K is 4.14×10^{-14} erg. For a whole unit of DNA, the density is 1.6 grams/cc the volume is 2.34×10^{-15}cc. So $\frac{n}{n_o} = e^{-(2.34 \times 10^{-15})(0.6)(980)(10^{-4})/4.14 \times 10^{-14}}$

$$= e^{-3.32 \times 10^{-3}}$$

This is very nearly $1-3.32 \times 10^{-3}$ or 0.997.

Thus the maximum difference in this statistical distribution introduced by gravity on what is easily the largest and most dense component in the cell amounts to less than three tenths of one per cent. It is very difficult to persuade oneself that a single bacterial cell, which is able to grow on agar where nutrient arrives from one side predominantly, or in solution, where it comes from all around, would be affected by a change from this statistical configuration to one in which n/n_o was exactly unity.

If we examine ribosomes (volume 4.2×10^{-18}cc, density 1.5 gram/cc) the modification is even more slight, being more like one part in a million. Therefore, we feel safe in concluding that change in the statistical distribution due to gravity plus Brownian

movement will not affect a single bacterial cell.

If next we examine a mammalian cell of 10 microns diameter, the situation is different. Here there are definite organelles, notably mitochondria. If we suppose the volume of one of these to be 10^{-12}cc and its density 1.1 grams per cc, then $n/n_o = e^{-2.4}$ or 0.09. This is a big difference. Removal of the gravity term would introduce a considerable change and would be expected to show in a definite way.

The nucleolus : probably the gravity receptor

Even more interesting is the nucleolus. This is, to a considerable part, comprised of RNA and can be supposed to have a density of 1.5 grams per cc on occasion, and also a volume of 10^{-12}cc. Substituting these figures in the formula for distribution with height, we reach the figure $e^{-11.8}$, or 7×10^{-6}. This is a very impressive difference and suggests that the nucleolus is the gravity receptor, if any one organelle plays that part.

The role of the nucleolus is not fully established. It seems likely that it is concerned with messenger RNA and thus with the actual creation of enzyme systems. A change in relative location of the nucleolus would thus be expected to produce a change in the formation of enzymes and hence, possibly of development.

Thus experiments with large cells, particularly those requiring vigorous respiration, or even better, those which can undergo development in some measurable way, would be expected to show definite effects of weightlessness. It should be remembered that,

in the ordinary way, there exists, under gravity, the mechanism of convection, which tends to mix up the material in a cell. So the above rather startling calculation is somewhat misleading, in view of a second effect of gravity; convection or streaming. We will defer consideration of convection for a moment.

Finally, if we examine systems of cells in layers, and if we suppose that objects as large as ribosomes pass from cell to cell so that they are subject to the statistical distribution of ribosomes between the top and bottom would be such that then for 100 microns, or one hundred cell layers, the distribution a 0.5% difference could exist. This might have an effect in that the removal of gravity would alter the distribution of ribosomes. However, it does not seem to suggest a striking effect.

Streaming due to density variation : convection.

The process of convection or an analogy to it is worth considering. If there is any advantage to a cell in using circulation as a means of providing synthetic regions with metabolites, then one would expect that evolution would have used that advantage. We can examine the sort of process which could use the variation of density in a gravitational field to secure a fresh supply of small molecules to feed a place where protein is being synthesized.

(a) Thermal Density Variation.

It is not at all easy to see that any appreciable temperature differences can be maintained in a cell. This means that any density differences could hardly be due to thermal reasons. To illustrate this point, consider the process of protein synthesis. Each peptide bond formed requires the contribution of 1/5 electron

volt (1500 calories per mole), to take an average estimate. Suppose that the energy were supplied by heat from the ribosome which is responsible for the synthesis. This small energy is of the right size for taking part in thermal processes (which are not normally used in biological systems), and might be thought of as cooling the ribosome while the action took place. Using the rapid synthetic rates of a bacterial cell, one finds that perhaps 100 peptide bonds could be made in a second. This is 2.4×10^{-19} calorie per second, and the mass of a ribosome is 4.15×10^{-18} gram. Assuming a specific heat of 0.5 calorie per gram, the temperature change is 0.1 degree per second. Using the thermal conductivity of water as 1.43×10^{-3} calories per sq. cm. per ^{o}C per cm., the temperature gradient needed to remove this amount of heat is $1.7 \times 10^{-4^{o}}$ C per cm. This is very small, and shows clearly that the process of temperature equalization on this scale is impressively fast.

"Simulation of Gravity Free Systems"

At this stage a word may be said on the process of simulation of the weightless condition by means of rotating systems which present multiple aspects to the earth's gravitational field. Such systems can be very successful in experiments where the rate of rotation is fast enough to bear some relation to the process of diffusion. If we are considering small molecules such as glycine, then the diffusion constant D is 9.6×10^{-6} square centimeters per second. Now we have for three dimensional diffusion, distance gone r in time t,

$$r^2 = 6Dt$$

So it can be seen that in one second the distance diffused is approximately 2/100 of a centimeter. If the system being studied is smaller than this, then it will have to be rotated more rapidly than 100 times a second in order to have a greater effect due to its aspect change than the effect due to the process of diffusion itself. Thus, once again, it does not seem sensible to make such studies with very small systems. On the other hand, larger systems such as developing plants will involve times of diffusion which are much longer than one second. And there the continued change of aspect would give real information.

Consumptive Density Variation

The process of random collision and specific selection which is probably responsible for the synthesis of protein and nucleic acid has the nature that the immediate vicinity of the point of synthesis is rapidly cleared of the colliding molecules. One estimate of the number of amino acids per cc needed to produce the necessary speed of protein synthesis is 3.5×10^{16}, or about 1/20,000 molar. If 100 molecules were removed per second then there could conceivably be a lack of solute in a small volume, and this small volume would thus have abnormally low density in a way which would be continually maintained. A guess at the volume is 3×10^{-15} cc per ribosome. If 1000 ribosomes were at work, the sort of figure treated as reasonable today, there would be a volume of 3×10^{-12} cc of abnormally low density. This figure is very probably large, as it exceeds the volume of a normal bacterial cell, but it can serve for an order of magnitude estimate

of the probable nature of convective circulation. For a density difference of 0.1, the upthrust due to this low density is 3×10^{-10} dyne. To try to estimate what kind of circulation might perhaps result, consider the very idealized case shown in the figure 1.

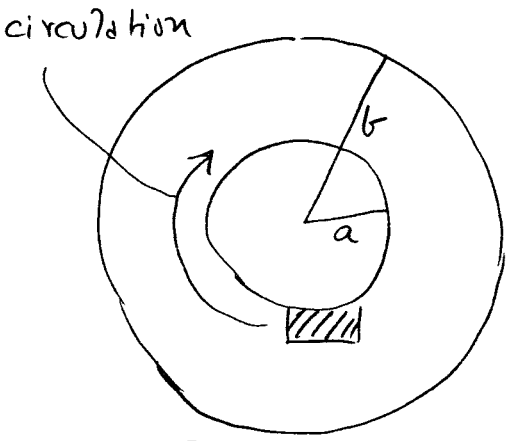

Figure 1

The synthetic region is supposed to be situated at the bottom of a cylindrical nucleus and to produce a region of low density at the bottom. This causes a tendency to rise and thus develop a circular motion which would bring new material into the synthetic region. An approximate estimate of the size of the motion can be obtained by assuming that the inner cylinder rotates around in a viscous medium. Applying the expression for the motion of two cylinders in a viscous medium, using inner and outer radii of 4,000 and 15,000A the relation for the angular velocity is Ω, where

$$\Omega = \frac{L}{4\pi\eta l}\left\{\frac{1}{a^2} - \frac{1}{b^2}\right\}$$

where L is the torque, in this case 1.2×10^{-15} dyne cm, η is the coefficient of viscosity, here taken to be unity, 1 the length of the cylinder, taken to be 10,000A and a and b are the inner and outer radii. The angular velocity calculated is 5×10^{-3} radians per second, or very roughly 3 revolutions per minute. The rate would be higher for a lower assumed viscosity, but measurements now being made on the viscosity of bacterial extracts indicate that it is higher than assumed (2). Thus such circulatory motion, which

has effectively been invented for the purpose of discussion, would not seem to be of much use to a small cell. The figure would be different in a large cell, for the possibilities of grouping a synthetic process in one place are greater and the viscosity is certainly less.

Thus the conclusion is reached that in a cell of dimensions about 10 microns across, with a cytoplasm of viscosity 0.1 poise or less, the process of synthesis will automatically produce regions of low density which will tend to rise in a gravitational field and so induce a streaming process. This streaming process will supplement diffusion and keep the cell more nearly in a condition where all components are equally distributed.

In this connection it can be seen why striking "zero g" effects are not expected for single cells even 10 microns in diameter. The reason is that while the statistical distribution of mitochondria, as described previously, may be such as to favor the lower regions of the cell under gravity, the metabolism of the cell will cause convective streaming which will tend to destroy the stratification. Thus two gravity induced effects tend to negate each other.

In any event, it is clear that for cells of 10 microns diameter and up, it is reasonable to expect some experimental findings from conditions of weightlessness.

Effect of stress on membranes

The existence of a common biological membrane for many types of cells is suggested by the measurements of membrane

capacitance, which all seem to give a figure of one microfarad per square centimeter; and electron micrograph studies. It seems reasonable to follow Danielli and suppose that the basic unit of a cell membrane is a pair of lipoprotein molecules and that the membrane is a two dimensional array of these.

There is also a limited amount of information on the surface tension of membranes. Figures run as low as 0.1 erg per sq. cm. A very interesting study by Weibull (3) on the effect of osmotic pressure on bacterial protoplasts makes it possible to estimate crudely the effect of stress on a membrane of the character found in bacteria. This will be discussed later.

If we consider the forces known to exist between macromolecules we find <u>two</u>. The first is the attraction due to London dispersion forces (Van der Waals forces), and the second is repulsion due to electrostatic charges. These charges are normally balanced to some extent by counter, or gegenions. In default of better information two plausible expressions for the potential energy of interaction have been supposed (4). For the London attraction between two cylinders of lipoprotein as seen in figure 2, where a is the radius and R is the <u>surface</u> separation, the potential energy U, in ergs, is (4)

$$U = 5 \times 10^{-13} a/R$$

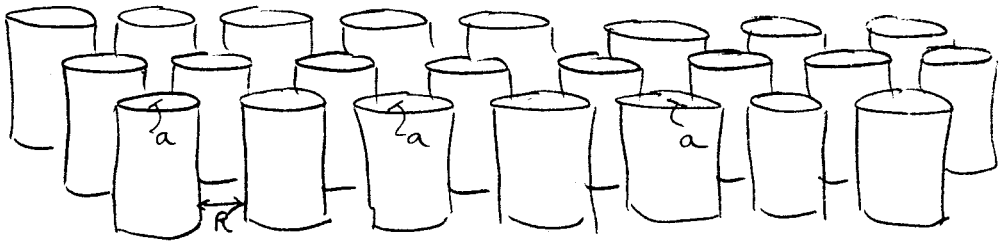

Figure 2

The repulsive potential U_r is of the form $U_r = Be^{-kR}$ (5) (greek "kappa")

where B is a very complicated function of the concentration (n per unit volume) and valence (v) of the ions, temperature and the electrical potential (ψ_0) at the surface of the molecules. If ψ_0 is taken to be about 30 millivolts, then the approximate value for U_r for two molecules, length 100A and width 10A is

$$5 \times 10^{-14} e^{-kR}$$ (greek kappa)

where k, familiar in the Debye-Huckel theory is

$$k = \frac{8\pi nv^2e^2}{\bar{K}kT}$$ (kappa)

where e is the charge on the electron and $\bar{K}$ is the dielectric constant.

When these two expressions are reduced to numbers it is at once clear that there are too many unknown factors to make a calculation of the potential between two lipoprotein molecules have much meaning. Thus there is no reasonable way in which, at

present, we can use these two basic physical interactions to predict the behavior of the membrane. We can, however, consider possible changes in the membrane and make an estimate of what they might produce in the way of altered passage of metabolites or hormones through the membrane.

Effect of Hydrostatic Stress

Now if a hydrostatic stress, due to gravity, is applied to the membrane, it will cause a distortion of the membrane. The simplest and best known distortion is the formation of a spherical bubble due to an internal pressure, p. The radius formed is r where $p = 2\ T/r$, T being the surface tension.

In a cell of 10 microns depth, the pressure on the lower membrane due to gravity is 10^{-3}g or approximately 1 dyne per cm^2. The membrane must be able to develop an internal strain so as to generate the opposing force to the hydrostatic pressure. This strain introduces a distortion, which involves an increased separation between the molecules. The molecules thus have less distance to move in order to permit a molecule such as (IAA) Indole acetic acid, to pass through. This means that a lower fluctuation is required and so might mean a greater membrane permeability.

While this theoretical suggestion is very hard to make quantitative, it is not hard to see that the hydrostatic pressure introduced by gravity probably <u>does</u> introduce a factor in permeability, though only for relatively large systems of cells, as in

a plant. We can make an estimate of the increase in membrane permeability as follows.

The pressure of 1 dyne per cm^2 on a membrane which has a bimolecular structure of thickness 100A means that a force must be developed within the membrane to withstand this pressure. Such a force will develop if the membrane stretches and thus develops an elastic force. The amount of distortion produced requires a knowledge of the elastic properties of the membrane. Weibull [3] has measured the change in volume of a protoplast of B. megaterium as the concentration of sucrose is changed. For a change in osmotic pressure of 4.2×10^5 dynes per cm^2 there is a change in volume from 2.7×10^{-12}cc to 2.0×10^{-12}cc. This corresponds to a change in area of 1.54×10^{-8} from an initial area of 9.3×10^{-8}cm. If we suppose that the tension "T" in the membrane is developed according to the familiar relation between pressure and tension in a spherical film, namely $p = 2T/r$ where p is the pressure and r is the radius we have, for these protoplasts $r = 8.5 \times 10^{-5}$cm, so $T = rp/2 = 18$ dynes/cm. This produces a change in area per original area of $1.54 \times 10^{-8}/9.3 \times 10^{-8} = 0.165$. We can thus calculate the Young's modulus of elasticity of the protoplast membrane, assuming it to be 100 Å thick. The force per unit area of cross section of membrane, the stress is 18 dynes/cm divided by 10^{-6}cm (100 Å) or 1.8×10^7 dynes/cm^2. So the stress divided by the strain is $1.8 \times 10^7/0.165 = 1.1 \times 10^8$ dynes/cm^2. This is a thousand times smaller than for a "weak" metal. The membrane does indeed yield easily.

If now we calculate the fractional change in area caused

by an end pressure of 1 dyne per cm^2 on a cell of width 10 microns, the force in the wall per unit length is the force on 10 microns square, or 10^{-6} dyne divided by the total side length of 40 microns or 2.5×10^{-4} dyne/cm. The stress is then $2.5 \times 10^{-4}/10^{-6}$ or 250 dynes/cm^2. The resulting change in area per unit area then becomes

$\Delta A/A$ = stress/Young's Modulus = $250/1.1 \times 10^8$

$= 2.2 \times 10^{-6}$ cm^2.

This is a small change and might not be expected to make an appreciable alteration in the permeability of the membrane.

A plant which is 1 meter high will exert a hydrostatic pressure on the lower membrane of 10^5 dynes/cm^2 in round numbers. Again in round numbers, this would cause a relative change of area of the lipoprotein membrane of 10^{-1}. This might well be sufficient to modify the permeability of a membrane, since it means that the average separation of a pair of lipoprotein molecules is increased by 30% The average separation of lipoprotein molecules can only be estimated, but assuming 5% of the dry weight of the cell to be lipid of molecular weight 1000, then for a double layer the average separation is close to 10A. An increase by 3A would not be negligible, especially as the surface separations must be less than the separation of the centers of the molecules. Thus the effect of gravity on the distribution of hormones which have to traverse membranes should become appreciable at plant sizes of 1 cm. and up.

The same general conclusion is reached for membranes with pores, although the kind of pore which results from a double lamella in the endoplasmic reticulum is hard to analyze.

Before leaving the subject of membranes, it can be pointed out that the number of membranes in a system of cells can be much larger than twice the number of cells. It is conceivable that a very small change in a large number of membranes could introduce a considerable action of polarization.

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SUMMARY

Small single cells should show little effect of weightlessness.

Cells as large as 10 microns across should show some effect due to the redistribution of mitochondria or the nucleolus. It is probable that if there is such a thing as a "gravity receptor" in a cell, it is the nucleolus.

Systems of cells in which relatively large objects, such as ribosomes, pass from cell to cell should show gravity effects.

Quite extensive systems, such as plants which exceed 1 cm in length should have increased membrane permeability at the lower end. This effect could be greatly increased if each cell had many internal membranes.

Experiments on weightlessness should also consider temperature as a variable.

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